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AN AIR-DRIVEN, AIR-FLOATED CAPILLARY TUBE ULTRACENTRIFUGE[1,2]

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An air-driven, air-floated ultracentrifuge of the Henroit and Huguenard type is described. The rotor is constructed with opposed radial holes in which glass capillary cells are inserted. The rotor may be stopped at appropriate intervals during the course of centrifugation, and the tubes removed and photographed. A simple optical system utilizing a horizontally mounted research microscope is used to obtain scattered light, absorption and schlieren pictures. Sedimentation velocity results are reported on earthworm blood, snail blood, and tobacco mosaic virus protein. The agreement with results reported, using other ultracentrifuges, is good. For tobacco mosaic virus certain exaggerated dilution effects at low concentrations are noted, probably attributable to the use of non-radial cells. This is not serious and may be taken into account. Although the rotor is run in air, the temperature at the position of the cell is essentially the same as the room temperature, and effects due to temperature gradients are negligible. Diffusion constants can be calculated from photographic schlieren records, of the spreading of centrifugally produced concentration gradients, made while the tube is mounted vertically on the microscope stage. Asymptotic packing volumes of the sedimented erythrocruorin of worm blood and of the colloidal proteins of milk can be measured from photographs of the cells after centrifugation at increasing centrifugal forces. The packing volumes agree satisfactorily with the voluminosities calculated from viscosity measurements using the Einstein equation. They also agree, for the colloidal milk proteins, with voluminosities calculated from analyses of deposits compacted in bowl rotors.

Introduction

The ultracentrifuge design and technique presented are based on the fact that liquid samples confined in sufficiently small cells are so completely immobilized that the centrifuge rotor can be repeatedly stopped and the cells removed for photographing without sensibly altering the sedimentation gradients. Cylindrical cells made from ordinary glass capillary tubing are used. The technique is simple and permits the ready measurement of sedimentation constants, diffusion constants, and voluminosities, all, if desired, on a single sample. Results for erythrocruorin of earthworm blood, hemocyanin of snail blood, and tobacco mosaic virus protein are here reported.

Immobilization of liquids during centrifugation by using capillary cells seems to have been described first by Elford.[3] Most of his cells were of metal. The progress of sedimentation was followed by bacteriological and chemical analyses of the contents of the cells after centrifuging for different times. Adaptations of this basic Elford analytical technique have been described by McIntosh and Selbie,[4] who, however, used cells actually of more than capillary diameter; by Polson[5]; by Ford and Ramsdell[6]; and by Brakke, Block and Wyckoff.[7] The last-named authors used long glass capillaries,

(1) Presented at the McBain Memorial Symposium, Colloid Section of the American Chemical Society, Chicago, September, 1953. The experiments on tobacco mosaic virus have, however, been added since.

(2) The work here described was done in the U. S. Department of Agriculture and supported in part by Bankhead–Jones Special Research Funds. Preliminary work was done previously by the senior author in the laboratory of the Shell Development Company, Emeryville, California.

(3) W. J. Elford, *Brit. J. Exp. Path.*, **17**, 399, 422 (1936).

(4) J. McIntosh and F. R. Selbie, *ibid.*, **18**, 162 (1937).

(5) A. Polson, *Nature*, **148**, 593 (1941).

(6) T. F. Ford and G. A. Ramsdell, "XIIth International Dairy Congress, Section II, Subject 1," Stockholm, 1950, p. 17.

(7) M. K. Brakke, L. M. Block and R. W. G. Wyckoff, *Am. J. Botany*, **38**, 332 (1951).

primarily for analysis, but they also observed the positions of concentration gradients by scattered light and calculated sedimentation constants from the data. McBain and Lewis[8] first reported optical measurements using capillary cells. They used thick-walled cells, inserted over pieces of photographic film in an air-driven spinning top type of ultracentrifuge. The rotor was run in dim light or under cover until a record was desired, whereupon the light was flashed on for one or more seconds. Ford and Ramsdell reported, but did not publish, optical measurements by the technique here described in 1946.[9] This work was referred to, and some of the pictures reproduced by McBain[10] as a part of a general discussion of ultracentrifugation. Recently Backus and Williams[11] have reported results obtained by centrifuging glass capillaries or capsules floated in a liquid of suitable density in an angle-head rotor. Subsequent to centrifugation the tubes are removed from the liquid, wiped dry, and examined by the schlieren method, making use of the optics of an electrophoresis apparatus and by differential absorption employing a spectrophotometer.

The present development has extended over many years. It has involved both improvement in operation of air-driven ultracentrifuges and development of techniques of handling and photographing capillary tubes. Our purpose has been to utilize the inherent simplicity and versatility of the air-driven spinning top. Our primary interest has been in colloids, and this is the reason for selection of the three particular test substances for which results are given in this paper.

The Centrifuge

Design of the Rotor.—A sectional sketch of a rotor is shown in Fig. 1. The outside diameter of this rotor is 8.26 cm. The rotor is supported and driven by a whirling sheet of air issuing from jets in a hollow cone stator. The principle of operation is that of the Henriot and Huguenard spinning top,[12] that of the early centrifuges built by Beams and co-workers,[13] and of the various centrifuges developed by McBain and co-workers.[10] However, the dimensions have been considerably increased over those used by these workers. In the rotor shown, the distance from the axis of rotation to the mid-point of the cell is about 3.25 cm., the same as in Svedberg's high-speed rotors.

Four pairs of holes for four diameters of tubes are equally spaced about the rotor, but only one tube is ordinarily used at a time. An opposed tube for balance is desirable. The holes are vented to the inside of the rotor to permit rapid equalization of internal air pressure as the rotor is stopped. Since air is thrown out of the rotor when spinning, a vacuum is produced, and without vents, as the centrifugal force drops to zero there is sometimes sufficient in-rush of air to blow the tubes out of the holes.

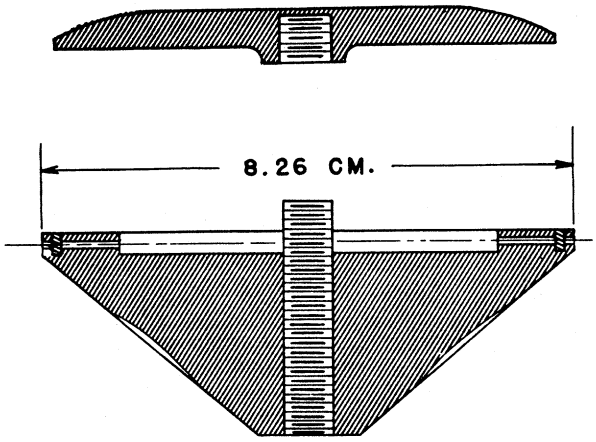

Fig. 1.—A capillary tube rotor.

Speed Control, Range and Measurement.—The speed of the rotor is determined solely by the setting of a valve at the centrifuge. The air is held in a large surge tank in the centrifuge room, and the pressure in the surge tank (usually 60 lb. per sq. in.) is controlled by a pressure regulator. The operation of the pressure regulator produces a fluctuation of about 0.4% in the r.p.s. over cycles of a few minutes, but the average speed remains constant within much closer limits for indefinite periods.

All of the sedimentation velocity experiments described in this paper were at relatively low speeds, 258 to 675 r.p.s. At these speeds, for the rotor shown in Fig. 1, the centrifugal forces at the mid-point of the cell are 8,720 and 59,700 times gravity, respectively. This rotor has been run at 960 r.p.s., giving a centrifugal force at the mid-point of the cell of 120,000 times gravity.

Below approximately 460 r.p.s. the speed is measured with an electronic tachometer and checked with a stroboscope. Above 460 r.p.s. a beat-frequency oscillator is used.

Temperature.—Experiments with thermocouples held close to the spinning rotor show the temperature at the upper surface at the mid-point of the cell to be about 0.02° below the room temperature of 258 r.p.s., 0.03° below room temperature at 480 r.p.s., and 0.03° below room temperature at 675 r.p.s. The corresponding temperature gradients along the tube in the direction of the centrifugal force are +0.11, +0.06, and −0.26°. The centrifuge is run in a temperature controlled room set at 19.5°. The normal fluctuation in room temperature is ±0.4° and the rotor temperature follows the room temperature.

The Capillary Tube Cell

The outside diameter of the capillary centrifuge tubes now regularly used is 1.59 mm., and the inside diameter about 1.10 mm. Although tubes of various lengths are used, a rotor with long holes being provided for long tubes, a good over-all length for use in the rotor shown in Fig. 1 is 13 to 14 mm. These tubes are made from selected

(8) J. W. McBain and A. H. Lewis, *Trans. Faraday Soc.*, **36**, 381 (1940); *Science*, **89**, 611 (1939).

(9) T. F. Ford and G. A. Ramsdell, paper presented before the Division of Colloid Chemistry of the 110th meeting of the American Chemical Society, Chicago, September, 1946.

(10) J. W. McBain, "Colloid Science," D. C. Heath and Company, Boston, Mass., 1950, pp. 228–230.

(11) R. C. Backus and R. C. Williams, *Arch. Biochem. Biophys.*, **49**, 434 (1954).

(12) E. Henriot and E. Huguenard, *Compt. rend.*, **180**, 1389 (1925); *J. phys. radium*, **8**, 443 (1927).

(13) J. W. Beams and E. G. Pickels, *Rev. Sci. Instru.*, **6**, 299 (1935).

micrometered sections of Pyrex capillary tubing. The tubes are always slightly tapered. The large ends are sealed in a tiny oxygen flame. Flat inside bases are produced, when desired, by inserting two or three Rose metal beads and then spinning the tubes immersed in hot lubricating oil, in special holders in a clinical centrifuge. Many tubes can be prepared in a short time. They are carefully cleaned and stored in vials. They can be re-used, and this is a desirable practice, since, in this way those with optical imperfections are gradually eliminated.

Tubes less than 1 mm. and greater than 1.59 mm. in outside diameter have been used. With large tubes, however, convection becomes a factor. Extremely small tubes are difficult to fill. The volume of liquid contained in a tube such as that described is less than 0.01 ml.

Optical Arrangements

The optical system, which need not be described in detail here, is essentially a photomicrographic apparatus. The centrifuge tube, which is mounted by means of a clip on a metal microscope slide (and on a rotatable insert for vertical alignment), replaces both the plane parallel cell and the cylindrical lens of standard schlieren systems. A good quality photographic enlarging lens mounted on the substage rack serves as the schlieren lens. The light source is an 8 v.-18 amp. tungsten ribbon filament lamp. Horizontal razor blades (for Toepler schlieren pictures), or inclined razor blades, wires, or spider webs (for Philpot schlieren pictures) are hung on a vertically adjustable crossarm just in front of the objective lens of the microscope. Wires and spider webs give well defined schlieren peaks, and razor blades give solid patterns which are easily observed visually. A collection of razor blades permanently set at various angles is provided. The apparatus is normally set to take one or another type of schlieren picture. Absorption pictures when desired require only that the razor blade be moved down, out of the light path. Scattered light pictures are obtained by switching off the schlieren light source, turning on a lamp which illuminates the tube from the side, and refocusing. Instant refocusing is secured by stops at both the schlieren and scattered light positions of the microscope tube. All of these types of pictures can be taken on the same tube in a few seconds. The enlargement used is about 6.6 times, for schlieren and absorption pictures. The image on the ground glass screen is reflected in a mirror for convenience. Cut film is used.

Operation

Filling and Sealing Tubes.—The liquid sample to be centrifuged is introduced into the tubes by means of a hypodermic syringe, and the length of the column adjusted to leave about 4 mm. of free space at the top of the tube. The filled tubes are sealed by spinning them immersed in barely molten petroleum jelly containing 0.5% sorbitan monostearate, in the clinical centrifuge, using the same holders used in melting the Rose metal beads. One or two minutes centrifuging is sufficient for this purpose. The petroleum jelly–sorbitan monostearate mixture gives an almost, though not quite, flat meniscus. Many mixtures of various substances have been tried for this purpose. The filled and petroleum jelly covered tubes are up-ended until ready for use, a practice which tends to erase any slight gradients produced by the clinical centrifugation.

Tubes have been sealed with white mineral oil also, which does not require any heating, and good results obtained despite considerable curvature of the meniscus.

Handling the Tubes.—When an experiment is to be started, the tube to be used is wiped clean, a little petroleum jelly removed from the top with a pin or sharpened toothpick to avoid overflow, and the tube again wiped clean with a soft cloth. Small metal racks are provided for tubes to be centrifuged and for those which have been centrifuged.

After the tubes are prepared, they are handled with tweezers. The rotor is marked so that when it is stopped, the tubes can be held upright during transfer to the metal microscope slide for photographing. The microscope slide is held in a vise for this operation. Although care is taken to avoid tilting the tubes during transfer, slight momentary tilting is in fact not serious. Shock does not appear to disturb the gradients, but they can be erased and homogeneous solutions again obtained by slowly up-ending, and gently rotating the tubes while they are held in a horizontal position, for about 20 minutes. Thus it is possible to rerun the same sample many times. It is found that clearer pictures and sharper gradients are often obtained on rerunning. This is partly due to clarification of the solution, and partly to further flattening of the meniscus.

Centrifugation.—The rotor with a tube in place is started by setting it on the air stream previously adjusted to the pressure desired. Acceleration is rapid. The final rotor speed is attained in about 90 seconds at 675 r.p.s., for example; in shorter times at higher speeds, and in longer times at lower speeds. The rotor is stopped by dropping the air pressure on a time schedule to 5 lb. per sq. in. and then braking with the fingers, although at low speeds it may not be necessary to reduce the air pressure. At 675 r.p.s. deceleration by dropping the air pressure requires a total of 58 seconds. For the acceleration and deceleration stages equivalent centrifuging times are calculated from speed–time plots. The combined acceleration and deceleration equivalent times may also be found by comparing results on the same sample run for different intervals.

The time elapsed in stopping the rotor, transferring the tube to the microscope slide, taking pictures, and replacing the tube in the rotor, averages about 3 minutes.

Measurements of Sedimentation Constants

The experiments on worm blood, snail blood, mixtures of worm blood and snail blood, and on tobacco mosaic virus are here reported in the chronological order in which the experiments were done. For the worm blood and snail blood experiments the rotor used was not the one shown in Figure 1, but a similar one of 7.62 cm. diameter.

Earthworm Blood.—The blood of the earthworm (*Lumbricus terrestris*) was chosen as a test substance because it is reported to contain a single respiratory protein, erythrocruorin, of moderate size[14] and because it is readily available, requires only clarification, and can be stored (at 4°) for periods up to 3 weeks or more. Total nitrogens on various lots of undiluted worm blood prepared by us were 1.53, 1.55, 1.68, 1.75, 1.86, 1.90, 1.96 and 1.97%. Of this total nitrogen, 84% is heat-coagulable, and about 87% is easily deposited by spinning at high speed in small air-driven bowl rotors. We have accordingly used the factor 0.85 for conversion of total nitrogen to pigment nitrogen. Analyses of worm blood samples variously depleted of erythrocruorin by centrifuging give the factor 5.75 for conversion of pigment nitrogen to erythrocruorin.

Figure 2 shows four types of sedimentation pictures for earthworm blood diluted with 0.9% sodium chloride solution to 0.168% total nitrogen or 0.82% erythrocruorin. These are all pictures of

(14) T. Svedberg and I. B. Eriksson, *J. Am. Chem. Soc.*, **55**, 2834 (1933).

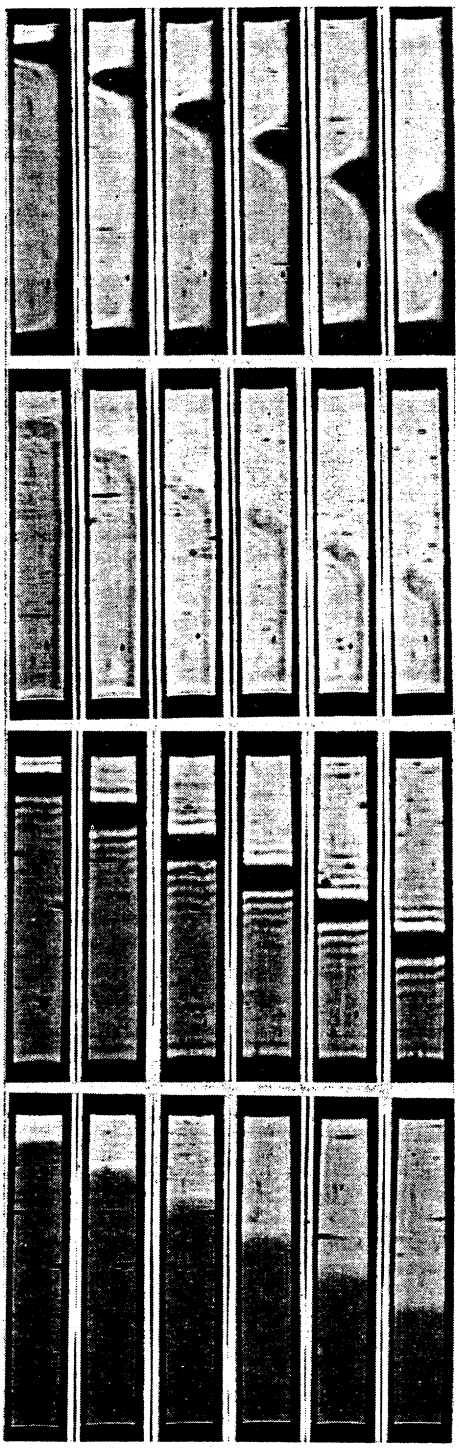

Fig. 2.—Various types of sedimentation pictures for earthworm blood. Earthworm blood diluted with 0.9% sodium chloride to 0.168% total nitrogen and 0.82% erythrocruorin; speed 485 r.p.s., centrifugal force about 27,500 times gravity; centrifuging times between pictures 8 min., equivalent to 511 sec. at 485 r.p.s. The top set of pictures was taken with an inclined razor blade (Philpot schlieren), the second set with an inclined 0.0005 inch diameter wire, the third set with a horizontal razor blade (Toeppler schlieren), and the bottom set with no razor blade (absorption). For the schlieren pictures panchromatic film was used; for the absorption pictures orthochromatic film. From top to bottom $s_{20} = 54.2$, 53.9, 55.5 and 54.0 $\times 10^{-13}$.

a single tube remixed and rerun over a period of 9 days. This particular tube was in fact rerun a total of 9 times in 20 days, with no significant change in the sedimentation constant. The tube was kept in a rack in the centrifuge room, at 19.5°, throughout this period.

Figure 3 is a plot of sedimentation constants, corrected to the standard state corresponding to water at 20°,[15] for earthworm blood at various concentrations in 0.9% sodium chloride. The effective centrifugal force in all of these experiments was about 27,500 times gravity. Eighteen runs were made at 0.168% total nitrogen (0.82% erythrocruorin), using four different tubes. The average of the 18 sedimentation constants obtained is 54.4 $\times 10^{-13}$, and the mean deviation from this average is 0.91 $\times 10^{-13}$, or 1.72%. On the main plot of Fig. 3 a median line is drawn through the value 54.4 $\times 10^{-13}$, and a deviation of $\pm 0.91 \times 10^{-13}$ is indicated by the dotted lines above and below this median line. From zero concentration to 0.210% total nitrogen the mean deviation of all the values is 1.25% of the sedimentation constant read from the median line. The intercept of the median line at zero concentration gives $s^{\circ}_{20} = 62.2 \times 10^{-13}$.

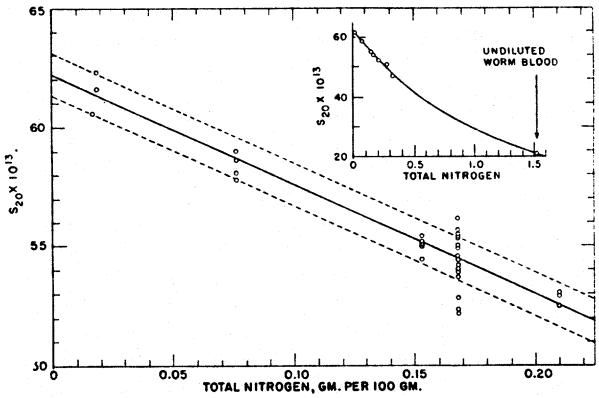

Fig. 3.—Relationship between sedimentation constant, corrected to the standard state, and total nitrogen concentration for earthworm blood diluted with 0.9% sodium chloride solution. The calculated concentrations of erythrocruorin range from 0.09 to 7.44%.

For *Lumbricus* erythrocruorin Svedberg and Eriksson[14] give $s_{20} = 60.9 \times 10^{-13}$ for worm blood diluted 25 to 30 times with 1% sodium chloride and with various buffers. The actual average of the fourteen values within the *p*H stability range used by them is, however, not 60.9 $\times 10^{-13}$ but 60.5 $\times 10^{-13}$. The mean deviation of their values from this average is 1.44 $\times 10^{-13}$, or 2.36%. They used a centrifugal force of 56,000 times gravity. Svedberg and Eriksson state that their undiluted blood contained over 4% erythrocruorin. On the basis of our analysis their diluted samples therefore contained at least 0.027–0.032% total nitrogen. Our value for s_{20} at 0.03% total nitrogen, taken from Fig. 3, is 60.8 $\times 10^{-13}$. The agreement between our values and Svedberg and Eriksson's is well within the limits of experimental error of both sets of data.

(15) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," The Clarendon Press, Oxford, 1940, pp. 34ff.

The sedimentation constants plotted in Fig. 3 are for each experiment averages of the sedimentation constants calculated for separate intervals. In most cases no effect of dilution due to centrifugation was noted, but in a few cases a definite increase in the sedimentation constant from the first interval to the last was observed. The total increase amounts to 2–3% of the mean sedimentation constant. Therefore, for strict accuracy, the various values and the final value, $s^{\circ}_{20} = 62.2 \times 10^{-13}$, should probably be diminished by about 1%.

Snail Blood (*Otala lactea*).—Figure 4 is a plot of sedimentation constants for the blood of *Otala lactea* at various concentrations at *p*H 4.26. The intercept at zero concentration gives $s^{\circ}_{20} = 91.7 \times 10^{-13}$. We know of no other published sedimentation results on the blood of this snail. The sedimentation constant obtained agrees satisfactorily with that found by Eriksson and Svedberg[16] for *Helix arbustorum*, but is lower than the values found by these authors for the hemocyanin of other snails.

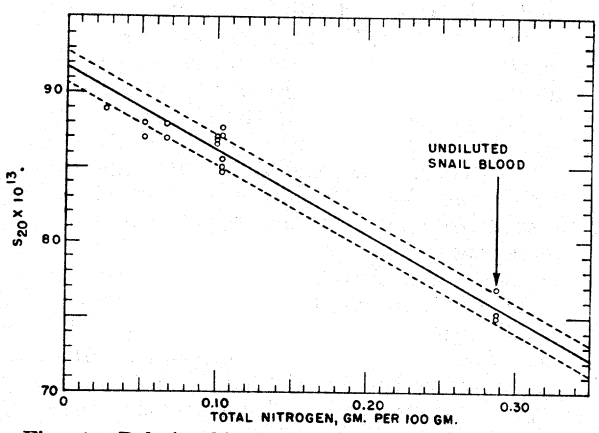

Fig. 4.—Relationship between sedimentation constant, corrected to the standard state, and total nitrogen concentration for snail blood (*otala lactea*) diluted with 0.2 *M* acetate–sodium chloride buffer at *p*H 4.26.

The blood of *Otala lactea* was also studied in buffers at *p*H 3.75, 6.63 and 8.20. In these solvents components having sedimentation constants agreeing with those of Fig. 4 were found, and in addition other components having sedimentation constants of about 50×10^{-13} and 20×10^{-13}.

Earthworm Blood–Snail Blood Mixtures.—A mixture composed of 50 parts of 0.186% total nitrogen worm blood in 0.9% sodium chloride, and 45 parts of undiluted snail blood containing 0.307% total nitrogen was prepared. Four tubes were filled with this mixture. These tubes were centrifuged on the first day and again, after standing (at 19.5°), four to five days later. The Philpot schlieren technique was used. On the first day a symmetrical lower peak and a slightly unsymmetrical upper peak were obtained in every case; on rerunning, both peaks were sharp and symmetrical (*cf.* Fig. 5). For the first day the pictures gave, for the lower peak, $s_{20} = 69.8 \pm 0.9\%$, and for the highest point of the upper peak, $s_{20} = 52.3 \pm 4\%$, $\times 10^{-13}$. The pictures obtained on rerunning the tubes gave $s_{20} = 77.0 \pm 1.4\%$ and $52.4 \pm 1.7\%$, $\times 10^{-13}$. The sedimentation constants of hemocyanin and erythrocruorin calculated for this mixture using the concentration plots of Figs. 3 and 4, are 76.9 and 52.2, $\times 10^{-13}$. Slow establishment of equilibrium is indicated. The dissymmetry of the upper peak observed the first day suggests the temporary existence of a degradation product of hemocyanin. The red color of erythrocruorin extended to the highest point of the peak.

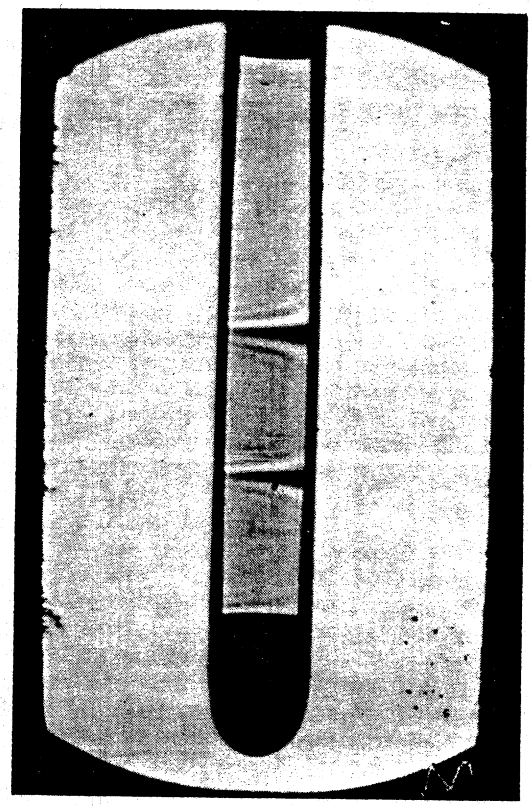

Fig. 5.—Philpot schlieren sedimentation picture for a mixture of earthworm blood and snail blood (*otala lactea*) in 0.9% sodium chloride solution for a tube rerun four days after filling. Worm blood total nitrogen, 0.098%; snail blood total nitrogen, 0.146%, speed 675 r.p.s.; centrifugal force about 56,500 times gravity; centrifuging time 24 minutes. $s_{20} = 52.0$ and 75.9×10^{-13}. This is a reproduction of the complete $2^1/_4$-inch $\times$ $3^1/_4$-inch negative.

Similar mixtures were made using undiluted worm blood, undiluted snail blood, and a 0.2 *M* phosphate buffer at *p*H 6.63. With these solutions no changes on standing for 3 to 4 days were observed. Both peaks were sharp and symmetrical, as in Fig. 5, on the first day and remained sharp. The sedimentation constants found were again in agreement with the calculated values.

Tobacco Mosaic Virus Protein.—The tobacco mosaic virus used was supplied by Prof. Max A. Lauffer. Analysis of the undiluted sample gave 0.297% nitrogen, 8.13% total solids and 1.095% phosphorus. The *p*H was 6.63. According to analyses by Best[17] tobacco mosaic virus protein contains 16.6% nitrogen and 0.52% phosphorus. Assuming all of the nitrogen in the sample received to represent tobacco mosaic virus, the concentration of virus was therefore 1.79%. The total solids

(16) I. B. Eriksson-Quensel and T. Svedberg, *Biol. Bull.*, **71**, 498 (1936).

(17) R. J. Best, *Australian J. Exp. Biol. Med. Sci.*, **26**, 65 (1948).

and phosphorus values indicate a rather high concentration of phosphate buffer probably containing some sodium or potassium chloride.

Various dilutions of this stock solution were prepared, using 0.1 M phosphate buffer at pH 6.61. Viscosities were measured, at 25°, on these solutions. These viscosities were divided by the viscosity of the 0.1 M buffer to obtain relative viscosities, η/η_0. The specific viscosities, $\eta/\eta_0 - 1$, are plotted against the concentration of virus protein in Fig. 6.

In all of the present experiments on tobacco mosaic virus except two, the centrifuge was run at 258 r.p.s., the average distance from axis to gradient was about 3.25 cm., and the average centrifugal force therefore 8,700 times gravity. The two undiluted samples centrifuged were run at 390 r.p.s. at an average centrifugal force of about 20,000 times gravity.

The results of all of the experiments on tobacco mosaic virus protein are presented graphically in Fig. 7. Some explanation of this plot is necessary. The reciprocals of the sedimentation constants are plotted against relative solution viscosities because Lauffer shows[18] that for tobacco mosaic virus, correction of observed sedimentation constants for solution viscosity tends to eliminate concentration effects. For Fig. 7 the observed sedimentation constants, at 19.5°, have been corrected to a buffer density of 1.011, the density of the 0.1 M buffer used for dilution. The density of the buffer or solvent in the original undiluted solution was taken to be 1.046. For the other solutions the densities were proportioned in accordance with the relative amounts of the original solution and added buffer. The partial specific volume used in these calculations was 0.73, which is that given by Lauffer.[18a] Some numerical error is possible as a result of these density corrections because of uncertainty as to the density of the original solvent. This error would be proportional to the concentration of virus, however, and would be zero at zero concentration.

In Fig. 7 the several sets of circles, squares and triangles represent separate experiments. For each experiment the reciprocal sedimentation constants calculated for each interval are plotted against the relative solution viscosities at the concentrations calculated to exist at the position of the gradient for the interval in question. These concentrations were found by multiplying the original concentration in each case by the square of the ratio of the distances from the center of rotation to the meniscus and to the position of the gradient. The relative viscosities were taken from the specific viscosity plot, Fig. 6. The method of calculating concentration is the same as used by Lauffer,[18b] and was for his centrifuge certainly justified since his cells were radial. To eliminate uncertainty in the squared radius relationship for cylindrical tubes, the interval s values for each set of experiments at the various calculated viscosities are connected by straight lines and extrapolated to the actual known viscosity of the solution at the original concentration. The intercepts are indicated by the arrows on the plot. These intercepts in turn define a curve which is drawn across the plot. At $\eta/\eta_0 = 1$, that is, at zero concentration of virus protein, the intercept gives $s_{obs.} = 187 \times 10^{-13}$. This is the observed sedimentation constant at zero concentration in 0.1 M phosphate buffer at 19.5°. Correcting to the standard state corresponding to water at 20° gives $s^0_{20} = 199 \times 10^{-13}$.

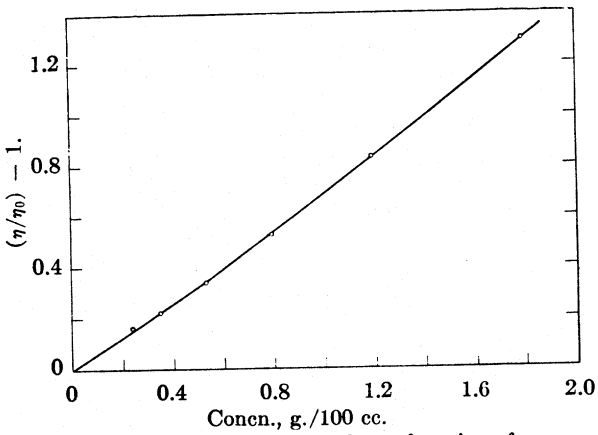

Fig. 6.—Specific viscosity plotted as a function of concentration for the tobacco mosaic virus protein preparation used in the present experiments.

Lauffer reports results of one experiment for which the effect of dilution during centrifugation was considered. In this experiment 33 pictures were taken and 32 sedimentation constants calculated. By multiplying each of these observed sedimentation constants by the corresponding relative solution viscosity Lauffer obtained a set of corrected sedimentation constants. Analysis of these values by the method of least squares gave $s_{obs.} = 215 \times 10^{-13}$. Correcting this result to the standard state corresponding to water at 20° gives $s^0_{20} = 198 \times 10^{-13}$. Since there was a drift in his set of values corrected for relative viscosity, Lauffer again corrected them, multiplying the observed sedimentation constants, not by the relative viscosities, but by hypothetical viscosities calculated by multiplying the intrinsic viscosity by the concentration. By this method he obtained $s^0_{20} = 187 \times 10^{-13}$. Lauffer reports also a set of sedimentation constants on another preparation at various dilutions. For these data the intercept of the $1/s$–concentration plot at zero concentration gives $s^0_{20} = 185 \times 10^{-13}$. These latter data were not corrected for dilution during centrifugation.

Wyckoff[19] gives sedimentation constants (s_{20} values) of 174 and 200 $\times 10^{-13}$ at 0.15% tobacco mosaic virus protein in 0.1 M phosphate buffer at pH 7. Assuming dilution according to the squared radius relationship, his actual average concentration would have been about 0.125%. At this concentration Figs. 6 and 7 give $s_{obs.} = 180 \times 10^{-13}$, and correcting this to the standard state gives $s_{20} = 192 \times 10^{-13}$. We have not observed two components in our experiments.

In Fig. 7 the straight lines through the points at the various concentrations, excepting at the lowest concentration, for which no line is drawn, were found by the method of least squares. These

(18) M. A. Lauffer, *J. Am. Chem. Soc.*, **66**, (a) 1188, (b) 1195 (1944).

(19) R. W. G. Wyckoff, *J. Biol. Chem.*, **121**, 219 (1937).

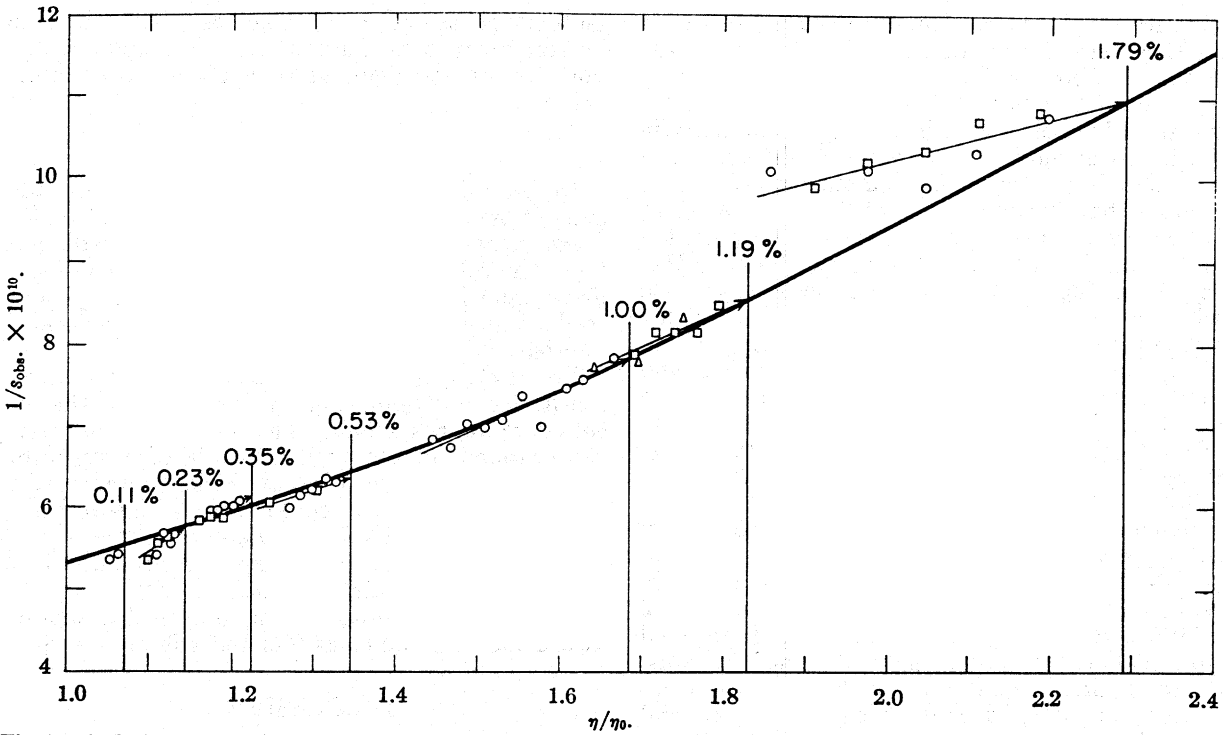

Fig. 7.—Relationship between observed reciprocal sedimentation constant at 19.5°, corrected to a buffer density of 1.011, and relative solution viscosity for tobacco mosaic virus protein.

straight lines parallel the curve through the intercepts only in the intermediate concentration range. The deviations in slope are consistent and we believe they are real. At the low concentrations they may indicate dilution in excess of that calculated using squared radius ratios. At the high concentrations the deviation is reversed and is probably here due to interference between particles during centrifugation, which would tend to neutralize the dilution effect.

The mean deviation of all of our values from the appropriate least squares values, that is, from the straight lines of Fig. 7, is 1.07%.

Measurement of Diffusion Constants

If a gradient is produced in a liquid column in a capillary cell by centrifugation, the necessary data for the calculation of the diffusion constant of the solute or dispersed substance can be obtained by taking Philpot schlieren pictures of the tube on the microscope stage at intervals as the gradient disappears. Five such sets of pictures for worm blood were taken using 0.9% sodium chloride solutions containing 0.82% erythrocruorin. These pictures gave $D_{obs.} = 2.0 \times 10^{-7} \pm 10\%$. The pictures were taken at ten-minute intervals and the experiments ran for 60 to 90 minutes. The diffusion gradient patterns were traced from enlargements of the negatives and also plotted from densitometer measurements across the negatives. Polson[20] gives $D_{20} = 1.81 \times 10^{-7}$ for *Lumbricus* erythrocruorin.

Measurement of Voluminosity

Use of the ultracentrifuge to measure the volume of sedimented influenza virus has been reported by Sharp, Beard and Beard.[21] They used a lucite cell 2 cm. long and 2 mm. in diameter in an air-driven vacuum type ultracentrifuge. The cell and the deposit or pellet in the bottom were photographed at frequent intervals over long periods at successively increased centrifugal forces. The authors note a swelling of the pellet when the centrifugal speed is quickly reduced from a high to a low value. This swelling is not rapid, however, amounting to about 8% of the volume in 15 minutes and continuing for several hours. We have made similar measurements of asymptotic packing volumes, but, as in the sedimentation experiments, photographed the tubes after stopping the rotor. For these experiments a 37 mm. diameter rotor was used. Since a rotor of this size can be stopped and the first picture taken in one minute, swelling is not great, and in any case the progress of swelling can be observed thereafter, and the volume extrapolated back to zero time. There are, moreover, advantages in being able to take schlieren, absorption or scattered light pictures at will during the swelling process.

The deposit from undiluted worm blood was compacted at gradually increasing centrifugal forces up to 240,000 times gravity over a period of 18 hours. The final volume of the deposit, corrected for swelling, divided by the weight of erythrocruorin present times its partial specific volume (0.740), gave an approximate packing volume or voluminosity of 3.0 ml. per ml. of contained dry protein. This total volume includes protein, water of hydration and solvate liquid. Ostwald viscosities were determined on various dilutions of the same blood. From a plot of these data the over-all volu-

(20) A. Polson, *Kolloid Z.*, **87**, 149 (1939).

(21) D. G. Sharp, D. Beard and J. W. Beard, *J. Biol. Chem.*, **182**, 279 (1950); D. G. Sharp, *Biochim. biophys. Acta*, **5**, 149 (1950).

minosity for the total protein calculated by use of the Einstein equation was 3.4 ml. per ml. Since the protein is about 85% erythrocruorin, the over-all voluminosity, thus calculated, cannot be far from the true Einstein voluminosity of erythrocruorin alone. Although in this case, for erythrocruorin—a small molecule compared to influenza virus—it is possible that packing was not complete even at 240,000 times gravity, the agreement between the values calculated by the two methods is nevertheless interesting. The progress of swelling of such jellies has been followed by successive schlieren pictures taken over periods of many hours. A distinct concentration gradient soon develops and moves upward until redispersion is almost or quite complete. Thus, although the swelling may be in part an effect of volume elasticity as stated by Sharp, *et al.*, diffusion processes are involved as well.

Similar compactions of the casein complex particles deposited from milk, which are very large, and for which an asymptotic volume was certainly reached, give for these particles a voluminosity of 4.3 ml. per ml. This value, for milk, is in good agreement with results of parallel compactions using an air-driven bowl rotor with which the compacted deposits could be removed for analysis, and with values calculated from viscosity measurements by ourselves and others. Since the casein complex particles are known to be spherical, the Einstein equation here certainly gives a true voluminosity, uncomplicated by shape factors. Thus, as concluded by Sharp, *et al.*, by centrifugal packing it is possible to express all the liquid not actually associated with the particles compressed. The method is therefore capable of giving a true measurement of voluminosity. For spheres the voluminosity is identical with the hydrodynamic volume.

Discussion and Summary

In the ultracentrifugal technique described the glass tubes or cells containing the material under investigation are cylindrical and of capillary diameter. The rotor into which they are inserted is air-driven and its rotation may be easily and quickly stopped at chosen time intervals for the purpose of photographing the tubes.

For the proteins used as test substances, good sensitivity and resolution are obtained and the results are reproducible. The sedimentation constants found for the erythrocruorin of earthworm blood and for tobacco mosaic virus protein agree satisfactorily with accepted values. Sedimentation constants for the hemocyanin of a snail blood not previously studied are consistent with published values for hemocyanins from other snails.

The technique provides simple optical means of measuring diffusion constants and voluminosities.

DISCUSSION

W. H. SLABAUGH.—With your equipment are you able to detect banding in the sedimentation of polydisperse systems such as observed in certain clay suspensions?

T. F. FORD.—We have observed banding with Bentonite. We have also observed banding in the casein-containing deposits from milk, and from milk containing worm blood as a reference substance. In the latter case a layer of the red worm blood erythrocruorin was deposited on top of the casein colloids.